

Method: 본 연구는 2000년 7월부터 2003년 8월까지 마리아 서울병원에서 IVF/ET program에 있던 환자들을 대상으로 하였으며, 환자의 나이는 32세에서 36세 사이로 환자간의 큰 차이는 없었으며, 이들에게 난자를 채취하기 36시간 전에 HCG를 모두 투여하였다. 난자를 채취하는 당일에 MII stage 난자가 한 개라도 있었던 환자군 (group I)과 MII stage 난자는 관찰되지 않고 미성숙 난자만이 있었던 환자군 (group II)의 난자의 성숙율, 수정율, 발생율 및 임신율을 조사하였다. 채취된 난자들을 성숙용 배양액 (YS with 30% HFF, 7IU/ml FSH, 1 IU/ml LH and 10 ng/ml EGF)에서 48시간 동안 배양하면서 0, 24, 48시간째에 그들의 성숙도를 관찰하고, 성숙된 난자들은 ICSI 방법을 이용하여 수정을 유도하였으며, 수정란은 성장용 배양액 (YS with 20% HFF)에서 5일 동안 배양하였다. 두 군에서 양질의 배아가 3개 이상이면 난자채취 6일째에 포배기 배아를 이식하였고 3개 이하이면 4일째에 cleavage stage의 배아를 이식하였다. 배아를 이식한 후 7주째에 G-sac이 확인되었을 때 임신으로 판정하였다.

Results: 채취된 난자의 수는 group 1에서 평균 16.4개였으며, 이는 group 2의 17.4개와 큰 차이를 나타내지 않았다. 성숙율을 확인한 결과 group 1과 2에서 각각 63.5%와 63.5%로 역시 두 군간에 차이를 확인할 수 없었다. 이어 수정율 (각각 79.2%와 79.4%)과 발생율 (각각 14.0%와 13.2%), 그리고 임신율 (각각 32.1%와 28.9%)에서도 역시 중요한 차이는 관찰되지 않았다.

Conclusions: 본 연구의 결과는 IVF/ET program에서 채란 당일에 MII stage 난자들이 관찰된다 할지라도 미성숙난자의 체외성숙, 체외수정 및 체외발생에 큰 영향을 미치지 않을 뿐 아니라, 그에 따른 임신율에도 영향을 미치지 않는다는 것을 시사한다. 그러므로 우량 난포가 존재한다 하더라도 미성숙 주기의 성공에는 별다른 영향을 미치지 않을 것이며, 우량 난포들이 관찰되더라도 IVF/ET program을 포기하지 않아도 되리라 사려된다.

P-59 Relationship between INSR, TNFR, PPAR- γ and PCOS in a Korean Population

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Background & Objectives: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. However, only a few genes have presented the relationship with PCOS. Receptors for insulin and insulin-like growth factor-1 have been found in ovarian tissues and several reports have shown consistent linkage and association with PCOS. TNFR2 mediates TNF- α actions and TNFR2 acts as a modulator. Variability at the gene encoding TNFR2 (TNFRSF1B, located at chromosome 1p36.2) also was reported that it has relationship with various symptom of PCOS. In addition, peroxisome proliferators-activated receptor- γ (PPAR- γ) mainly expressed in adipose and has been thought to be involved in the obesity. It also reported that relationship between PPAR- γ and insulin resistance in several studies. Because one of the representative symptoms for PCOS is obese, relationship between the PPAR- γ -

polymorphism and PCOS is under investigation. Currently, studies of Pro12Ala variant in exon2 and C/T substitution in exon6 in PCOS patients showed that the levels of BMI and leptin were correlated with polymorphisms of exon6. In this study, we investigated the polymorphism of these three genes to determine whether they are associated with PCOS in Korean women of reproductive age.

Method: Using restriction fragment length polymorphism (RFLP), the polymorphisms were analyzed in 71 Korean PCOS patients and in 26 control patients.

Results: MM type of TNFR2 exon6 (69%) and CT type of INSR (48%) were predominant than other types (TNFR2; MR, RR, INSR; CC, TT). In PPAR- γ studies, the C/G polymorphism of exon2 was similar to previous reports, however, that of exon6 showed that the C/T phenotype was predominant than C/C type, which is different from Italians.

Conclusions: Interestingly, the predominance for CT type of INSR was not shown with a Korean population, different from the previous report done by other research groups in the United States. We also observed the different predominance of polymorphisms in exon6 against previous report.

P-60 A Simple, Easy and Efficient Vitrification Method for Cryopreservation of In Vitro Produced Human Embryos

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Background & Objectives: This study was to examine the usability of a new vitrification method for cryopreservation of in vitro produced human embryos from IVF-ET program.

Method: Human multi-pronuclear (>3PN) embryos were co-cultured with cumulus cells in modified CR1aa medium for 5 to 6 days. In vitro developed blastocysts were collected and exposed in vitrification solution with 2-steps; i) 10% ethylene glycol (EG) and 10% FBS in D-PBS for 5 min. ii) 30% EG, 0.5 M sucrose (S) and 10% FBS in D-PBS for 30 sec. And then embryos were loaded onto our designed minimum volume cooling (MVC) straw and plunged directly into LN₂. For thawing, MVC straw in LN₂ was quickly transferred into 0.5 MS (and 10% FBS in D-PBS). After recovery, embryos were again transferred into 0.25 MS, 0.125 MS and then finally into 10% FBS in D-PBS for 1 min per each. All treated embryos were transferred onto cumulus cell drop in 10% FBS added CR1aa. 16 hrs after thawing, embryo survival was determined and stained with hoechst 33342 or Live and Dead reagent to check the viability.

Results: By simple and easy vitrification and thawing method (freezing for 6min.; thawing for 5 min.), survival of human embryos was indicated high percentage (79.2%, n=24). Also, when optically survived embryos were determined by Live and Dead staining (live=green color, dead= red color), almost of them were confirmed viable.

Conclusions: Therefore, using this introduced vitrification method, human embryos can be cryopreserved with simple and efficient.